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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/751,235	01/02/2004	Dean DellaPenna	MSU-08604	3881

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EXAMINER	
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ART UNIT	PAPER NUMBER
1638	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/751,235	DELLAPENNA ET AL.	
	Examiner	Art Unit	
	CATHY K. WORLEY	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 July 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-8,10-17 and 21-36* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-17 and 21-36* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. The amendment filed July 1, 2008, has been entered.

2. Claims 9, 18-20, and 37 have been cancelled.

Claims 1-8, 10-17, and 21-36 are pending and are examined in the present Office Action.

3. The text of those sections of Title 35, U.S. Code not included in this office action can be found in a prior office action.

Objections and Rejections that are Withdrawn

4. The objection to the title is withdrawn in light of the Applicant's amendment of the title.

5. The objections to claims 22 and 33 are withdrawn in light of the Applicant's amendments to the claims.

6. The rejections of claims 33, 35, and 37 under 35 USC 112, second paragraph, are withdrawn in light of the Applicant's amendments to the claims.

7. The rejection of claims 1-8, 11-17, 21-25, and 30-36 under 35 USC 112, first paragraph, for lack of written description is withdrawn in light of the Applicant's amendments to the claims and after further consideration in light of the new Written Description guidelines published in 2008 online at http://www.uspto.gov/web/menu/written.pdf).

8. The rejection of claim 37 under 35 USC 112, first paragraph, for lack of enablement is withdrawn in light of the Applicant's cancellation of the claim.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

9. Claims 11-13, 16, 17, 21-30, and 32-34 are rejected under 35 U.S.C. 101 because the claimed invention lacks patentable utility.

The claims are directed to a nucleic acid encoding a protein that is capable of complementing the mutant phenotype of the Arabidopsis *lut1* mutant. This is interesting from a basic science perspective, but it is not a substantial utility. The resulting plant is a transgenic *lut1* mutant Arabidopsis plant with a wild-type phenotype. This is a very expensive wild-type-phenotype weed. There is no evidence that one of skill in the art can engineer a transgenic plant with a useful phenotype by using the claimed nucleic acids to express the claimed proteins.

The Applicant has argued that the nucleic acids of the instant invention can be used as probes to isolate homologs (see declaration by Dr. DellaPenna, submitted on Feb. 7, 2008). This is not a specific utility, however, because anyone of the known cytochrome P450 genes could be used as a probe to identify homologs.

For these reasons the claims lack a substantial, specific utility.

Claim Rejections - 35 USC § 112

10. Claim 36 remains rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for the reasons of record stated in the previous Office Action mailed on Aug. 7, 2007. The Applicant's arguments in the response filed on Feb. 7, 2008, were fully considered but were not found to be persuasive.

Claim 36 recites the limitation "The plant tissue of Claim 34" in line 1. There is insufficient antecedent basis for this limitation in the claim.

The Applicant states that they have amended claim 35 to provide antecedent basis for claim 36 (see third paragraph on page 10 of the response). This is not persuasive, however, because claim 36 depends from claim 34 rather than claim 35.

11. Claims 1-8, 10-17, and 21-36 remain rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement for the reasons of record stated in the previous Office Action mailed on Aug. 7, 2007. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Applicant's arguments in the response filed on Feb. 7, 2008, were fully considered but were not found to be persuasive.

Claims 1-8, 10-17, and 21-36 are broadly drawn to expression vectors, nucleic acids, transgenic plants and seeds, and methods comprising a nucleic acid sequence encoding a polypeptide at least 72% identical to SEQ ID NO:4 and having monoxygenase P450 activity.

The nature of the invention is molecular biological approaches for using a nucleic acid discovered by complementing a mutant.

The specification discloses that a nucleic acid comprising SEQ ID NO:5 was identified by its ability to complement the *lut1* mutation in *Arabidopsis* (see page 102 lines 6-9 and Figure 19a). This nucleic acid encodes the amino acids identified as SEQ ID NO:4 (see Figure 19a). A subsequence of SEQ ID NO:4 is identified as SEQ ID NO:1 (see Figure 18). The specification discloses that bioinformatics analyses suggests the polypeptide of SEQ ID NO:4 is a cytochrome P450 enzyme and comprises an oxygen binding pocket consensus sequence (SEQ ID NO:12), a

heme-binding cysteine motif (SEQ ID NO:14), a chloroplast targeting peptide (SEQ ID NO:11), and a transmembrane domain (SEQ ID NO:10), (see pages 102-103 and Figure 22).

The specification does not disclose any enzyme assays showing that the protein encoded by SEQ ID NO:5 has a specific enzymatic function. Transformation of the *lut1* mutant *Arabidopsis* plant with SEQ ID NO:5 complements the mutant phenotype and therefore, either directly or indirectly, provides β-ring hydroxylase and ε-ring hydroxylase activity (see page 103-104 and Figure 17). However, subsequent experimental work was unsuccessful in providing an assay for enzymatic function (see Tian et al PNAS (2004) Vol. 101, pp. 402-407). Tian et al teach that initial attempts to express and assay LUT1 protein in yeast were unsuccessful (see Tian et al, page 405, left column), and expression in bacteria is highly unlikely to work given the problems of expression eukaryotic membrane proteins in prokaryotic systems (see Hannig et al TIBTECH (1998) Vol. 16, "focus", see second-to-last page, right column). Therefore, one of skill in the art would not know how to use the nucleic acids and vectors for prokaryotic or yeast expression (claims 11 and 14 are specifically not enabled for these reasons).

The instant application speculates that SEQ ID NO:5 encodes a cytochrome P450 enzyme with β-ring hydroxylase and ε-ring hydroxylase activity that is involved in carotenoid biosynthesis, however, even if this hypothesis is true, multiple enzymes are involved in this pathway, and it is highly unpredictable what

phenotype would result from overexpression of only one of the enzymes involved. The prior art teaches that metabolic engineering of biosynthetic pathways is highly unpredictable (see Stephanopoulos et al TIBTECH (1993), Vol. 11, pp. 392- 396). It is possible the required enzymes may have to be present in stoichiometric quantities, or there could be feedback regulation mechanisms that are complex. It would require undue experimentation on the part of one of skill in the art to determine the results of expressing SEQ ID NO:5 in a plant, and to elucidate what other steps (if any) would be required to generate a useful plant.

Given this unpredictability and given that the specification in the instant application has not provided any working examples of expression of SEQ ID NO:5 in a healthy wild-type plant to demonstrate there is an effect on carotenoid metabolism (other than complementing a mutant which is deficient in the identical enzyme), one of skill in the art would not know how to use the claimed expression vectors, nucleic acids, transgenic plants and seeds, and the methods recited in claims 28-32 are not enabled.

POTENTIAL SCOPE OF ENABLEMENT

Even if the Applicant can provide support for a use of SEQ ID NO:5, the enablement would not be extended to the entire genus of molecules encompassed by these claims. The claims encompass nucleic acids encoding polypeptides with as little as 72% identity to SEQ ID NO:4, and one of skill in the art would not know how to use any such nucleic acids. The specification does not disclose any nucleic

acid other than SEQ ID NO:5 that encodes a polypeptide at least 72% identical to SEQ ID NO:4 that has been shown to have the function of producing zeinxanthin and the function of complementing the *lut1* mutation in *Arabidopsis*, and there are multitudes of nucleic acids encompassed by this recitation. Even if there were some guidance on how to use a nucleic acid encoding ε-ring hydroxylase and β-ring hydroxylase activity that is involved in carotenoid biosynthesis, there is no guarantee that one of skill in the art would be successful in expressing a polypeptide with as little as 72% identity to SEQ ID NO:4 to produce a recombinant protein with monoxygenase P450 activity. The specification has not provided any working example to demonstrate that a polypeptide having as little as 72% identity and comprising SEQ ID NO:1, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:14 would be capable of catalyzing any reaction at all. These domains, put together, have not been shown to be sufficient for enzymatic activity or the desired carotenoid biosynthesis function.

Given the breadth of the claims, the unpredictability in the art, and the lack of working examples, it would require undue experimentation on the part of one of skill in the art to make and use the invention as claimed.

APPLICANT'S ARGUMENTS AND AFFIDAVIT

The Applicant has submitted a declaration signed by one of the inventors, Dr. DellaPenna (see Affidavit submitted on Feb. 7, 2008). In this declaration, the inventor states that one of skill in the art could identify proteins that are at least

72% identical to SEQ ID NO:4 by complementation of LUT1 mutants (see part 2 of the declaration). This is not persuasive, however, because identifying a protein that can complement an Arabidopsis mutant does not necessarily mean the protein will be expressed successfully in any other heterologous system (such as E. Coli or yeast); and it does not show that a transgenic plant expressing the protein will have any phenotype other than wild-type. Therefore, one of skill in the art would not know how to use this protein or nucleic acid encoding the protein for anything other than complementing a mutant Arabidopsis plant, which is not a substantial utility (see rejection under 35 USC 101, above).

Also in the declaration, Dr. DellaPenna states that in post-filing art, Quinlan et al successfully expressed a rice homolog in E.coli.; and this rice homolog is the instant SEQ ID NO:16 and has 78% identity to SEQ ID NO:4 (see part 3 of the declaration). This is not persuasive, however, because the Applicant elected SEQ ID NO:4 for prosecution, the Applicant did not elect SEQ ID NO:16 for prosecution. This is one example of the unpredictability in the art, because the Applicant could not successfully express SEQ ID NO:4 in yeast, but in post-filing art, a different investigator was successful in expressing SEQ ID NO:16 in E. coli. Even at the time of the publication by Quinlan et al which was more than 3 years after the filing of the instant application, Quinlan et al state that only a subset of P450 enzymes function in E. coli and the heterologous bacterial system is not necessarily a feasible approach (see page 148, left column). Furthermore, Quinlan et al utilized a

complicated system of expressing SEQ ID NO:16 in a pCOLADuet vector in BL21 (DE3) cells along with a second plasmid encoding the Arabidopsis lycopene ε cyclase (see page 148, right column). The instant specification did not provide this type of detailed guidance about how to successfully express a monooxygenase in E. Coli; and Quinlan et al did not publish their work until after the instant Application was filed. Therefore, the instant specification did not provide enablement for successful expression of SEQ ID NO:4 in bacteria or yeast.

Also in the declaration, Dr. DellaPenna states that the Arabidopsis coding sequence (SEQ ID NO:5) can be used to identify a homolog, such as the rice homolog (SEQ ID NO:16) which was shown to be active in E. Coli (see part 3 of the declaration). This is not persuasive, however, because using SEQ ID NO:5 as a probe to find homologs is not a specific utility (see rejection under 35 USC 101, above). Any cytochrome P450 could be used as a probe to find other P450s, therefore, this is a throw-away utility that is not specific to the elected sequence of SEQ ID NO:5.

In the remarks filed on Feb. 7, 2008, the Applicant argues that E. coli expressing the rice homolog with hydroxylase activity demonstrates that the enablement requirement was met (see paragraph bridging pages 13-14 of the response). This is not persuasive, however, because the Applicant elected SEQ ID NO:4 for prosecution, the Applicant did not elect the rice homolog (SEQ ID NO:16) for prosecution. This is one example of the unpredictability in the art, because the

Applicant could not successfully express SEQ ID NO:4 in yeast (see lines 5-6 on page 103 of the instant specification), but in post-filing art, a different investigator was successful in expressing SEQ ID NO:16 in E. coli. Even at the time of the publication by Quinlan et al which was more than 3 years after the filing of the instant application, Quinlan et al state that only a subset of P450 enzymes function in E. coli and the heterologous bacterial system is not necessarily a feasible approach (see page 148, left column). Furthermore, Quinlan et al utilized a complicated system of expressing SEQ ID NO:16 in a pCOLADuet vector in BL21 (DE3) cells along with a second plasmid encoding the Arabidopsis lycopene ε cyclase (see page 148, right column). The instant specification did not provide this type of detailed guidance about how to successfully express a monooxygenase in E. Coli; and Quinlan et al did not publish their work until after the instant Application was filed. Therefore, the instant specification did not provide enablement for successful expression of SEQ ID NO:4 in bacteria or yeast.

It is noted that the Applicant has not provided any arguments about the unpredictability of expressing one component of a metabolic pathway in an attempt to increase the yield of a product of the pathway. This is the main issue with regard to enablement of the instant invention. In the instant invention, the Applicant is attempting to modify carotenoid biosynthesis by expressing one enzyme. However, multiple enzymes are involved in this pathway, and it is highly unpredictable what phenotype would result from overexpression of only one of the enzymes involved.

The Examiner emphasizes that the prior art teaches that metabolic engineering of biosynthetic pathways is highly unpredictable (see Stephanopoulos et al TIBTECH (1993), Vol. 11, pp. 392- 396).

For the reasons stated above, it would require undue experimentation on the part of one of skill in the art to determine the results of expressing SEQ ID NO:5 in a plant, and to elucidate what other steps (if any) would be required to generate a useful plant.

12. All claims remain rejected.

13. The Applicant was invited to submit evidence of successful expression of the elected sequence in the Office Action mailed on Dec. 18, 2006. The Applicant is invited, again, to submit evidence of successful expression of the polypeptide of SEQ ID NO:5 in yeast or E. coli; and evidence of successful alteration of carotenoids in a non-mutant transgenic plant expressing the polypeptide of SEQ ID NO:5.

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Cathy K. Worley whose telephone number is (571) 272-8784. The examiner has a variable schedule but can normally be reached on M-F 10:00 - 4:00 with variable hours before 10:00 and after 4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Marie Grunberg, can be reached on (571) 272-0975. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Cathy K. Worley/
Patent Examiner, Art Unit 1638